

## REMARKS

## Claim Status

Claims 1, 6-8, and 25-30 are pending in this application. Claims 2-5 and 9-24 have been withdrawn from consideration. Claims 25, 27 and 29 are currently amended. New claim 31 has been added. No claim is canceled

Applicants note the withdrawal of the rejection over Li et al., *Proc. Natl. Acad. Sci. USA*. 1997; 94: 73-78.

### **Rejections Under 35 U.S.C. §112-Indefiniteness**

Claim 29 stands rejected as indefinite for reciting the phrase “a predetermined biological.” The Examiner contends that this is vague and indefinite since the adjective “biological” does not modify a noun.

To address this rejection, the word “function” has been inserted following the term “biological” in claim 29. Accordingly, withdrawal of this rejection is respectfully requested.

### **Rejections Under 35 U.S.C. §112-Written Description, New Matter**

Claim 25 stands rejected for allegedly lacking sufficient written description, and containing new matter not described in the specification for the terms “identifying thermal  $\beta$ -factors, using calorimetric values from thermodynamic studies, or using computer simulation algorithms.” The Examiner contends that the specification only describes thermal  $\beta$ -factors that are identified from NMR signals, or microcalorimetric analysis, which the Examiner interprets as being different from the claimed limitations.

This rejection is respectfully traversed. The Examiner is partially correct in his interpretation that thermal  $\beta$ -factors are identified from NMR images. However, thermal  $\beta$ -

factors are also identified from crystal structures, or by a computer algorithm. As discussed with Examiner Ly and Examiner Marschel during the interview in September 2004, identifying the functionally critical sites and/or allosteric cavities *may* be identified using NMR and crystal structure imaging, but the method does not rely on such imaging being available due to the sophisticated computer modeling that was available and widely used by those skilled in the art as of the filing date of the present application. Accordingly, the present claims encompass practicing the claimed method on proteins for which such NMR or crystal structure imaging is available, but does not depend on the structures being imaged using NMR or crystallization.

It is further noted that the specification discloses using calorimetric values from thermodynamic studies as an independent method, see page 10, line 18 which states that cavities can be identified using NMR, crystallization “...or microcalorimetric analysis of complex or mutation analysis of molecule...” (emphasis supplied). This method does not depend on prior identification of NMR or crystal structure imaging. In contrast, it is the use of  $\beta$ -factors that is used **if** the target protein is structurally imaged using crystallization or NMR. Claim 25 has been amended, and new claim 31 has been added to reflect this distinction.

To expedite prosecution, claim 25 has been amended to recite that the identification of the allosteric cavity is achieved using nuclear magnetic resonance, crystal structure analysis, or computer modeling. Support for this amendment can be found in the specification at page 11, lines 3-5. New dependent claim 31 has been added. This claim specifies that when the cavity is identified by NMR or crystallization, then the method further comprises identifying thermal  $\beta$ -factors, using calorimetric values from thermodynamic studies.

In view of the above, withdrawal of this rejection is respectfully requested.

### **Rejections Under 35 U.S.C. §102(a)-Anticipation**

Claims 1, 6-8, and 26-30 stand rejected as anticipated by an abstract to Aghajari et al. *Protein Science*. March 1998; 7: 564-72 (“Aghajari”). The Examiner contends that Aghajari describes a method for identifying a compound that is an allosteric modulation of the alpha-amylase enzyme, by Aghajari’s disclosure of a chloride binding site that is located 5 angstroms from the active site (i.e., the substrate binding site). According to the Examiner’s interpretation, the chloride-binding site has the dimensions and properties of the allosteric cavity as presently claimed.

This rejection is respectfully traversed for the following reasons. First, in order for a single reference to be an anticipating reference under 35 U.S.C. §102(a), the reference must expressly or inherently disclose every limitation of a claim with sufficient precision and detail to establish that the subject matter claimed existed in the prior art. *Rowe v. Dror*, 112 F.3d 473 (Fed. Cir. 1997); and *Verve, LLC v. Crane Cams, Inc.*, 311 F.3 1116 (Fed. Cir. 2002).

Second, while Applicants do not dispute that chloride binding site that is located 5 angstroms from the active site (i.e., the substrate binding site) is an allosteric site on  $\alpha$ -amylase, the present claims are not directed to the well-known technique of allosteric modification. The present specification explicitly discloses that allosteric modulation is well-known for both enzymes and receptors (see page 4, lines. 18-21). Neither are the present claims directed to a method of determining *whether* proteins are already allosterically modified, or to a method of identifying compounds other than known allosteric modulators that bind to a protein in a known allosteric cavity. The present Applicants readily concede that they did not discover the existence of allosteric cavities, or the concept of allosteric modification.

By contrast, the present inventors discovered, and the present claims are directed to, a method of identifying a compound that is an allosteric modulator of a protein by first identifying a candidate allosteric cavity on a target protein that is a measurable distance from the functionally

critical site of the target protein, in order to find a compound that is an allosteric modulator of interactions at the functionally critical site between the target protein and a modifier (e.g., ligand or co-factor etc.), i.e., the present inventors found a new way to use the properties of allosteric cavities, and the known concept of allosteric modification, in a method for screening for compounds that are allosteric modulators of a protein. Implicit in the claims language is the concept that, in order to carry out the claimed step of identifying a *candidate* allosteric cavity, such a cavity must not be already known to be an allosteric site of the protein-of-interest (or it would not be a “candidate” cavity). In addition, where the target protein-of-interest is already known to be allosterically modified, and thus, necessarily has an allosteric cavity, the claimed step of identifying a candidate allosteric cavity would apply to a cavity **distinct** from the known allosteric cavity. Thus, the present invention teaches how to modulate normal functional protein (which may or may not bound cofactors or natural allosteric modulators) by targeted allosteric modulation.

Aghajari does not disclose the presently claimed method, much less each and every limitation of the present claims, as is required for an anticipation rejection under 35 U.S.C. §102(a). Aghajari discloses the structure and activity an  $\alpha$ -amylase enzyme from a psychrophilic bacterium that lives in the Antarctic as determined by crystallization. According to Aghajari, a chloride ion, which functions as an allosteric co-factor, is required for activity of this  $\alpha$ -amylase (page 564, col. 2, ll. 6-10). Co-factors are non-protein substances that help an enzyme to carry out its catalytic action. Cofactors may be cations, such as the chloride ion disclosed by Aghajari, or organic molecules known as coenzymes. Aghajari clearly states that binding of co-factor is required for enzyme function. Otherwise, the enzyme remains as an inactive apoenzyme. The role of cofactor is to enhance the enzyme's rate of catalysis. Aghajari did not discover, and does not disclose, that binding chloride ion or any other cofactor *modulates* enzyme function. Rather, it is a condition precedent for enzyme function.

As further support for the contention that Aghajari did not “identify” a candidate allosteric cavity, it is noted that Aghajari discloses that the presence of the chloride ion as a co-factor is a property of many chloride-dependent  $\alpha$ -amylases (page 568, col. 1, second full ¶). The Aghajari abstract explicitly discloses that the  $\alpha$ -amylase enzyme identified from *Alteromonas haloplanctis* has significant similarity to the mammalian  $\alpha$ -amylases. The full article corresponding to the Aghajari abstract (attached as Exhibit 1) specifically discloses that it has long been known that mammalian  $\alpha$ -amylases require a chloride ion as an allosteric activator (page 564, col. 2, lines 6-8; and page 568, col. 1, second full ¶). In fact, the article cites a paper from 1971 (Thoma et al.) which demonstrates that the skilled artisan knew that  $\alpha$ -amylase was allosterically modified at least 33 years ago. Moreover, the Aghajari paper discloses that mammalian salivary  $\alpha$ -amylase was crystallized in 1991 (see citation to Ramasubbu et al., 1991 on page 565), and the structure of a porcine  $\alpha$ -amylase was refined and published in 1994, including the precise location of the chloride-binding allosteric site (see citation to Larson et al. on page 565; see also citations to Qian et al. and Feller et al. on page 568).

Thus, in contrast to the present claims, Aghajari does **not** disclose a method of screening for allosteric modulators (i.e., identifying a compound that is an allosteric modulator of interactions at the active site), much less by the specific steps recited in the present claims, i.e., identifying an candidate allosteric cavity on a target protein that is a measurable distance from the functionally critical site of said target protein;

calculating the dimensions of said cavity and mapping the chemical and/or electrostatic properties of said cavity;

identifying compounds that contain functional groups that can be accommodated by said cavity;

and testing said compounds in an *in vitro* assay to detect a compound which binds within the cavity of said target protein and allosterically modulates intermolecular interactions at the functionally critical site between said target protein and said modifier.

In contrast to the present claims, Aghajari describes determining the crystal structure of a bacterial  $\alpha$ -amylase not previously characterized (see Materials and Methods, page 571). It is emphasized that the native, naturally-occurring  $\alpha$ -amylase as crystallized in Aghajari is *already* bound to its allosteric modulator (the chloride ion; see Figure 4 on page 567). In fact, a subsequent publication by Aghajari (*Protein Science*. 2002; 11: 1435-41; "Aghajari 2"; attached as Exhibit 2), states that that it was not possible to obtain crystals of an  $\alpha$ -amylase that is not bound to a chloride ion, because it is too unstable. As indicated above, the chloride ion is a necessary cofactor for the enzyme that is required for enzyme function.

Accordingly, the Aghajari teaching of characterizing the structure of a protein already known to be allosterically modulated, and already bound to its allosteric modulator does not disclose any step of the method of the present claims.

Continuing the analysis, step (a) of the present claims, "identifying an allosteric cavity on a target," where the cavity is a *candidate* site, is not disclosed by Aghajari since  $\alpha$ -amylase was already known to be allosterically modified at that site. As discussed on page 12 of this paper, the present claims do not encompass identifying an allosteric site that is already known to exist and is *necessary* for the protein's function. A site that is necessary for the function of the protein, such as the site on the  $\alpha$ -amylase that binds to the chloride ion, is an "active site" according to the present specification, and is not a candidate allosteric site which must be distal from the active site.

In addition, in order to carry out the step of "identifying" a candidate allosteric site, such a site must not already be known to be an allosteric site (otherwise it would already be identified). Except for crystallizing a previously un-crystallized  $\alpha$ -amylase enzyme from a psychophilic

bacteria, Aghajari does not disclose identification of any candidate allosteric (or other) cavities that have not been previously characterized on  $\alpha$ -amylases. To the contrary, Aghajari explicitly discloses that this  $\alpha$ -amylase displays the same overall fold as *all*  $\alpha$ -amylases described so far (page 565, col. 1, lines 1-3), binds a calcium ion about 12 angstroms from the active site as do *all*  $\alpha$ -amylases (page 565, col. 1, lines 20-24), and has a highly conserved active site containing three essential amino acids as do *all*  $\alpha$ -amylases (page 565-566, bridging sentence; see also Figure 6). Accordingly, Aghajari does not disclose identifying any previously uncharacterized “candidate” allosteric site as called for in the present claims.

Step (b) of the present claims, calculating the dimensions of the cavity and mapping the chemical and/or electrostatic properties, also is not disclosed by Aghajari. As indicated above, Aghajari discloses that the bacterial  $\alpha$ -amylase has the same allosteric cavity as all known  $\alpha$ -amylases, and that previous work by others already characterized the amino acids critical for binding the chloride ion (i.e., the chemical properties), and the electrostatic properties of the known allosteric cavity. See page 568, col. 1 to 2. Aghajari’s re-stating of known chemical and electrostatic properties of a known allosteric cavity is not encompassed by step (b) of the present claims, which is directed to characterizing properties of a *candidate* allosteric cavity, i.e., a cavity that likely may be an allosteric site but which is not yet characterized or determined to be such a site.

Step (c) of the present claims, identifying compounds that contain functional groups that can be accommodated by the identified allosteric cavity, is certainly not disclosed by Aghajari. As indicated above, Aghajari 2 states that that it was not possible to obtain crystals of an  $\alpha$ -amylase that is not bound to a chloride ion, because it is too unstable. Except for the chloride ion, the known allosteric modulator bound at a known allosteric site, there is no teaching or suggestion in Aghajari of any method of identifying compounds that contain functional groups that can be accommodated by the previously known allosteric cavity, much less a previously uncharacterized allosteric cavity as called for in the present claims.

Step (d) of the present claims, testing the compounds (that were identified by screening) in an *in vitro* assay to detect a compound which binds within the cavity (identified by screening) and allosterically modulates the target protein similarly is not disclosed by Aghajari. Since Aghajari does not disclose identifying allosteric modulators, but only confirming the presence of a known allosteric modulator bound at a known allosteric site, Aghajari certainly does disclose testing any such non-existent compounds in assays.

Moreover, the only modulation of the  $\alpha$ -amylase enzyme disclosed in Aghajari (as called for in the present claims) is inhibition by Tris, a *known* inhibitor of  $\alpha$ -amylases (see page 569, col. 1, lines 1-4). The inhibition by Tris is via competitive binding of one molecule of Tris in the active site (i.e., functionally critical site) of the  $\alpha$ -amylase (see page 569, col. 2, ll. 8-12). Competitive inhibition by binding in the active site and preventing the enzyme substrate from binding is not even allosteric modulation. Similar to the  $\alpha$ -amylase-chloride ion crystals isolated in complex with each other, the Tris-  $\alpha$ -amylase interaction also was crystallized as complex, i.e., Tris was added to the buffer from which the crystals were made. This by no means constitutes a method of screening to identify compounds that bind in a candidate allosteric site and modulate interactions at the active site (i.e., functionally critical site) as called for in the present claims.

In addition, the only “searching” to identify sites on the  $\alpha$ -amylase enzyme disclosed in Aghajari is in the context of searching for “catalytic residues” as found in proteases, in order to confirm that the enzyme has autoprolytic activity. Clearly this identification of catalytic residues has nothing to do with identifying a candidate allosteric cavity or identifying compounds that bind in the newly identified allosteric cavity as potential allosteric modulators of a protein, as called for in the present claims.

A further difference between Aghajari’s disclosure of  $\alpha$ -amylase crystallization and present claim 31 is that the only thermal  $\beta$ -factor determination in Aghajari is within the active site of the enzyme or over the entire structure (page 571, col. 1, ll. 7-15). Aghajari does not disclose the presently claimed step of using the thermal  $\beta$ -factor determination to identify an candidate



allosteric cavity proximal to the active site using thermal  $\beta$ -factor determination as called for in claim 31.

### **Conclusion**

In conclusion, the present claims do not embrace the well-known concept of allosteric modification, much less allosteric modulation of enzymes already known to be allosterically modulated by ions, such as  $\alpha$ -amylases. Rather, the present claims call for a method of identifying compounds that are allosteric modulators of proteins by first identifying candidate allosteric sites, i.e., sites not previously known to be allosteric sites.

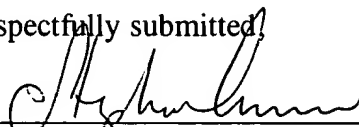
Aghajari does not anticipate the present claims because Aghajari does not disclose or suggest any screening method to identify candidate allosteric cavities and modulators that bind in such cavities, much less the specific steps defined by the present claims. Aghajari discloses crystallization of an  $\alpha$ -amylase *already* bound to its known allosteric modulator, at an allosteric site which is conserved in  $\alpha$ -amylases from several different species. This method is unrelated to the claimed method of identifying novel allosteric modulators of proteins by first identifying proximal cavities to the active site (functionally critical site) as candidate allosteric cavities, and then characterizing the cavity's chemical et al. properties, and then screening for compounds that bind in the cavity **and** allosterically modulate the cavity, as set forth in the present claims.

In view of the above amendment, Applicants believe the pending application is in condition for allowance.

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